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INCREASING WHITE ASH SEED GERMINATION BY EMBRYO DISSECTION

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ABSTRACT.--Dissection of white ash embryos from the seed, coupled with cytokinin treatment, has been shown to enhance germination and seedling survival, as compared to whole seed stratification methods.

OXFORD: 161.6:176.1 *Fraxinus americana*.
KEY WORDS: zeatin, stratification, viability, yield, treatment.

A major barrier to the mass production of some forest trees is seed dormancy. Dormancy may be imposed by mechanisms ranging from impermeable seed coats to hormonal inhibitors (Leopold and Kriedemann 1975). Once the dormancy mechanism is known for a particular species, it can be circumvented, often by mimicking some environmental influence. In the laboratory, dormancy can often be broken with growth stimulants.

Seeds of white ash (*Fraxinus americana* L.) normally are dormant after imbibition (Mayer and Poljakoff-Mayber 1963). Sondheimer *et al.* (1968) demonstrated that dormant seeds of this species contain high concentrations of the potent germination inhibitor, abscisic acid (ABA), in both the embryo and endosperm. Stratification can break dormancy in white ash seeds, apparently both by reducing the ABA level (Sondheimer *et al.* 1968) and by stimulating the appearance of growth stimulators (Villiers and Wareing 1965), probably hormones, in the embryo. On the other hand Sondheimer and Galson (1966) and Tzou *et al.* (1973) have induced dormant ash embryos (not stratified) to germinate by treating them with hormones (especially gibberellins and cytokinins). However, at best only about 30 percent of the seeds can be induced to germinate by either of these methods (McBride and Dickson 1972).

We used this information to design a system for germinating large numbers of ash embryos. We found that removing the embryos from the seeds and treating them with growth hormones gives much higher germination rates than conventional seed handling techniques.

METHODS

Seed Viability

About 400 seeds were sliced longitudinally and placed in a 1 mg/ml solution of tetrazolium red in 0.1 M phosphate buffer, pH 7.0 for 6 hours. Viable embryos were stained red (Burstone 1962). Nearly 97 percent of the seeds were viable. Several imbibing techniques were employed (including a cold treatment) but no difference in apparent viability was found.

Embryo Germination

Preliminary germination tests were carried out with several hormones, to find the best germination stimulator. Embryos were treated with zeatin, kinetin, and gibberellic acid, at concentrations reported by Sondheimer and Galson (1966), and Tzou, *et al.* (1973) to be effective in promoting germination of white ash seeds. Test procedures was as follows.

Winged seeds showing no insect damage were collected from several trees. The seeds were soaked in water for 24 hours, removed from the wings, and soaked for an additional 48 hours. The embryos were carefully dissected from the endosperm. They were placed in petri dishes, on filter paper soaked with hormone solution prepared in 0.01 M potassium phosphate buffer, pH 6.0, 10 embryos per dish. The dishes were then kept either in a growth chamber with a 17-hour day, or in a greenhouse with continuous illumination provided by supplemental fluorescent lighting.

After 10 days the germination rate was determined, and germinated embryos were transplanted to sand in styrofoam cups, which were capped with petri dish covers to prevent dessication. The survival rate was recorded at 13 days and again at 30 days after the start of the experiment.

A major factor reducing viability in the embryos was damage inflicted during dissection. Damaged embryos rapidly become necrotic and failed to germinate.

Only zeatin proved effective in stimulating ash embryo germination (fig. 1). Based on this result, extensive tests were done on germination and survival rates of ash embryos with zeatin.

Stratification

Seed was planted in moist sand, placed in an incubator (68-78°F) for 30 days, transferred to a cooler (38°F) for 30 days, then allowed to germinate in an incubator for 60 days. Germination counts were made every two days.

RESULTS

The results (table 1) show that the embryo dissection technique can lead to much

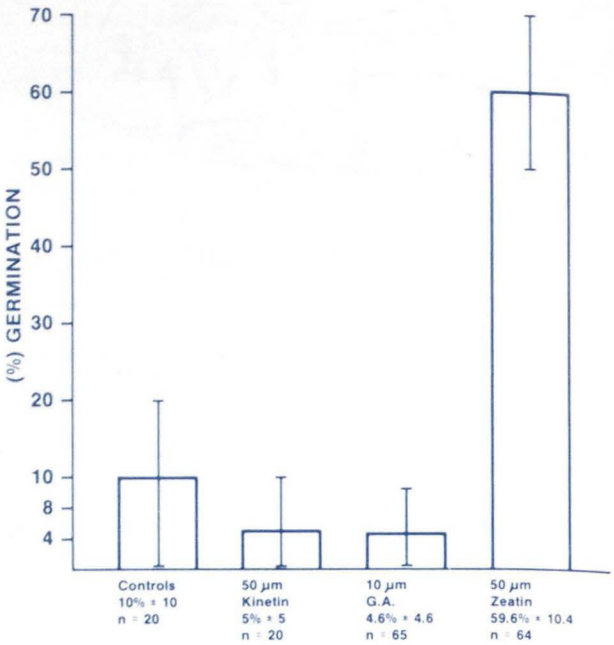

Figure 1.--*Results of preliminary germination tests with several hormones. Error bars are two standard deviations.*

greater germination than can stratification methods. Comparing germination and survival rates of hormone-treated embryos with those for untreated embryos in the two growth conditions indicates that environment may influence germination rates. Under the conditions used, continuous light appeared to produce much the same effect as the zeatin treatment. Apparently the environment also interacts with the genome of the seedling. The zeatin-treated embryos of the tree #13 had a low survival rate in the greenhouse, but not in the growth room; while the situation is reversed for the seeds of tree #14.

At the end of the 30-day growth period in sand in styrofoam cups, all the surviving trees were well developed and growing vigorously. They had one to two sets of true leaves and extensive root systems. The overall length of the plants from the various treatments was not significantly different. The mean length was 62.3 mm (S.E. = 4.15). These trees could be transplanted to soil and raised to maturity.

DISCUSSION

The embryo dissection technique appears to be a valuable tool to circumvent the problems of germinating dormant ash seeds.

Table 1.--*Results of germination tests on zeatin treated (+Z) control (H_2O) embryos and whole seed stratification. The embryo method is based on 12 observations from 4 trees and 3 dishes. Stratification results are from 400 seeds per tree.*

(In percent)

Tree	Greenhouse				Growth Room				Stratification germination
	Germination		Survival		Germination		Survival		
	+Z	H_2O	+Z	H_2O	+Z	H_2O	+Z	H_2O	
2	100	100	97	97	100	70	100	70	24
7	100	67	100	67	100	20	100	20	12
13	97	93	77	93	100	73	100	73	39
14	100	97	100	93	97	70	77	70	72
Mean	99	89	93	87	99	58	94	58	37
$s_{\bar{x}}$	.8	7.6	5.6	6.9	.8	12.7	5.8	12.7	13

Survival rates above 90 percent are 2 to 3 times greater than obtained by stratifying and planting whole seeds.

This technique may be useful for selecting desired seedling phenotypes. Light and/or temperature conditions can be altered to select seedlings with increased chances of survival in particular natural conditions. In addition, the high rates of survival help to maximize yields when the number of seeds is limited, such as controlled crosses of particularly desirable seed parents.

In general, this technique provides a rapid and reliable method to obtain ash seedlings at a much higher yield than has been previously possible.

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